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(54) Title: METHODS FOR ENGINEERING ARTIFICIAL VETO CELLS

(57) Abstract

A method for producing an artificial veto cell capable of specifically inhibiting the proliferation, cytotoxicity or cytokine secretion or inducing apoptosis or necrotic cell death of a T-cell directed to alloantigen or processed antigen comprising the steps of providing an antigen presenting cell having the alloantigen or processed antigen, and externally contacting the extracellular surface of the antigen presenting cell with a chimera comprising a non-CD8 polypeptide capable of reducing T-cell proliferation, cytotoxicity or cytokine secretion or inducing T-cell apoptosis or necrotic cell death and a moiety sufficient to bind the chimera to the surface of the antigen presenting cell in a manner which presents the polypeptide on the cell's surface such that the polypeptide is able to reduce T-cell proliferation, cytotoxicity or cytokine secretion or induce T-cell apoptosis or necrotic cell death.

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DESCRIPTION

METHODS FOR ENGINEERING ARTIFICIAL VETO CELLS

Field of the Invention

This invention relates to methods and reagents for engineering artificial veto cells ("AVCs") for purposes of immunotherapy.

Background of the Invention

The following is a general discussion of relevant art, none of which is admitted to be prior art to the invention.

Antigen-specific (hereinafter referred to as "specific") immunotolerization is a therapeutic endpoint in subjects in need of the selective suppression of untoward immune responses to defined antigens. Given the centrality of pathogenic T lymphocytes in autoimmune, alloimmune, and other acute and chronic inflammatory diseases, an objective in the field of immunotherapy is the development of strategies for inhibiting specific T-cells. Antigen-presenting cells ("APCs") such as dendritic cells, B lymphocytes and macrophages provide one efficient means for accessing antigen-specific T-cells (T lymphocytes).

In general, APCs are responsible for initiating most immune responses through their pivotal role in antigen presentation to T-cells. During antigen presentation, endogenously processed nominal antigen peptides associate intracellularly with either class I major histocompatibility complex ("MHC") heterodimers or with class II MHC heterodimers, and the resulting heterotrimeric complexes are then translocated to APC surfaces (reviewed in Germain and Margulies, 11 Annu. Rev. Immunol. 403, 1993; Germain, 76 Cell 287, 1994). For a single immuno-

genic protein that is pulsed with APCs, processing frequently yields a diverse MHC:nominal antigen peptide repertoire, the composition of which may differ between different APCs (Falk et al., 348 Nature 248, 1990; Falk et al., 174 J. Exp. Med. 425, 1991; Henderson et al., 255 Science 1264, 1992; Henderson et al., 90 Proc. Natl. Acad. Sci. USA 10275, 1993; Hunt et al., 255 Science 1261, 1992; Rammensee et al., 11 Annu. Rev. Immunol. 213, 1993; Huczko et al., 151 J. Immunol. 2572, 1993).

In order to achieve a more defined MHC:nominal antigen peptide repertoire on a given APC surface, it is experimentally feasible to load exogenously synthesized oligopeptides onto class I or class II MHC heterodimers (Harding et al., 86 Proc. Natl. Acad. Sci. USA 4230, 1989). Such exogenously-loaded peptides do not require intracellular proteolytic processing.

For purposes of antigen presentation, it is common to employ one of two categories of cells, that is, cells that naturally express MHC heterodimers and cells transfected with an MHC gene expression cassette. In principle, another category of an MHC-bearing cell is one in which the MHC molecule has been exogenously attached to the cell surface. A method for delivering an MHC molecule to an APC surface has been described which entails the use of an HLA-A2.1-streptavidin chemical conjugate which can be added to a pre-biotinylated cell (Elliott and Eisen, 87 Proc. Natl. Acad. Sci. USA 5213, 1990). The chemical moiety was shown to be alloantigenic, but no data was presented that it can bind and present a nominal antigen peptide.

Functional interactions between APCs and T-cells are known to be mediated by both cell surface-associated and soluble molecules. Antigen-specificity in this interaction is provided by the major histocompatibility complex:nominal antigen peptide complex of the APC interacting with the T-cell receptor (TCR) of the T-cell,

constituting a trimolecular axis. However, effective interactions additionally require certain cell surface-associated and soluble costimulator molecules of APCs binding to receptors for these costimulators on T-cells. Examples of known costimulators on APCs are B7 (Linsley et al., 87 Proc. Natl. Acad. Sci. U.S.A. 5031, 1990); ICAM-I (van Seventer et al., 144 J. Immunol. 4579, 1990); VCAM-I (van Seventer et al., 174 J. Exp. Med. 901, 1991); LFA-3 (van Seventer et al., 21 Eur. J. Immunol. 1711, 1991; fibronectin (Shimizu et al., 145 J. Immunol. 59, 1990; Nojima et al., 172 J. Exp. Med. 1185, 1990; Davis et al., 145 J. Immunol. 785, 1990).

Tykocinski and Kaplan, U.S. Patent No. 5,242,687 disclose a method for effecting conversion of a T-cell activator into a T-cell inhibitor by expressing a CD8 polypeptide on the surface of an APC. In this case, the CD8 molecule functions as a "coinhibitor", triggering an inhibition program within the T-cell.

Summary of the Invention

The present invention provides different methods and compositions for molecularly engineering APCs to tailor their T-cell modulatory properties, for both in vivo and ex vivo applications. Applicant has determined a broad set of methods for converting APCs from T-cell activators into T-cell inhibitors. An APC functioning in an T-cell inhibitory mode can be referred to as either a "deletional APC" (since it is deleting a specific T-cell through either the induction of anergy or apoptosis) or a "veto cell" (since it is vetoing a specific T-cell in a manner that mimics the natural veto function of an ill-defined mononuclear cell population in the periphery). Previously, CD8 coating was the only known method for engineering AVCs. The present invention offers a series of additional methods for generating non-naturally occurring veto cells, establishing the concept that "artificial

veto cells (AVCs)" constitute a broader class of cells that can be purposefully engineered through diverse methods.

A key aspect of the present invention is that the APC is being used as a delivery vehicle for an inhibitory reagent. The APC provides an efficient means for specific T-cell targeting, thereby focusing the reagent upon specific T-cell responders that are engaged by the antigenic peptides presented at the APC surface. Hence, the antigen presentation function of the APC is being used to advantage. By this approach, the inhibitory molecular reagent can be delivered locally, bypassing the need to administer it systemically and thus avoiding potential toxicities associated with systemic administration. Thus a critical aspect of the present invention is that the T-cell modulator is acting locally, either through cell-to-cell contact or local transit.

Another advantage is that the inherent capacity of the APC to process and present diverse antigenic peptides is being exploited. This obviates the need for prior knowledge of the precise antigenic peptides within a complex protein mixture, such as a cell extract, that are responsible for T-cell pathogenesis. AVCs can thus be pulsed with extracts from target tissues of a given disease, and the AVC (via its APC function) can present the diverse repertoire of potential pathogenic peptides to the T-cell population of the patient.

Applicant has determined those molecules that can be newly expressed on or in APCs in order to convert them into AVCs. Reagents useful for AVC engineering include both cell surface-associated and soluble molecules. Applicant believes that in each instance an inhibitory reagent provided by the APC binds to a receptor for the reagent on the T-cell, and this binding event serves to trigger an inhibitory program within the T-cell.

Applicant herein discloses that any one of a

number of molecules with known T-cell inhibitory activity can be used as a reagent for AVC engineering. Agents known to induce apoptosis in T-cells or other types of cells constitute candidates. A preferred polypeptide for AVC generation is Fas ligand ("Fas-L") (also known as APO-1 ligand) which is known to be capable of inducing apoptosis in both resting and activated T-cells via signaling through Fas (the Fas-L receptor, Fas antigen) molecules on the T-cell surfaces.

Other T-cell apoptotic pathways can be utilized as well. For example, it is known that soluble antibodies with specificity for either the class I MHC heavy chain or the class I MHC β_2 microglobulin light chain can inhibit the proliferation and triggering of cytotoxicity of T-cells, and it is further believed that in at least some contexts such antibodies evoke T-cell apoptosis. Hence, according to the present invention, another preferred reagent is an anti-class I MHC antibody or an anti-β_2 microglobulin antibody expressed by or on the AVC. Alternatively, to avoid having to use heterodimeric antibody molecules and to thereby simplify the application of the approach with a smaller reagent, one can generate single chain anti-class I Fvs that incorporate the heavy and light chain variable regions obtained from the corresponding polypeptides into a single polypeptide chain. There are numerous examples for the successful production of soluble functional Fvs directed against other target molecules, and these provide clearcut guidelines for the design and production of functional Fvs. Both cell surface-associated and soluble forms of the antibodies or Fvs can be employed.

In addition to known apoptosis inducers, other molecules with T-cell inhibitory potential can be employed for AVC engineering. Such molecules may either act directly on the T-cell or may modulate other molecular systems of the AVC itself, conferring or enhancing the T-

cell inhibitory action of the AVC. Preferred reagents in this category include known immunosuppressive cytokines, for example, interleukin-10 ("IL-10"), transforming growth factor β, heterodimeric placental protein 14, homodimeric/monomeric placental protein 14, and viral proteins. Examples of an immunoregulatory viral gene products include those originating from the human immunodeficiency virus (HIV). One example of a HIV immunoregulatory protein is tat, but HIV immunoregulatory proteins are not limited to tat. These various proteins share in common T-cell inhibitory activity, though they differ in their respective inhibitory mechanisms.

Applicant discloses that AVC engineering can be accomplished through both gene and protein transfer. Gene transfer is especially well-suited for expressing immunosuppressive genes in AVCs that encode for the production of secreted proteins, but is obviously also applicable to the expression of cell surface reagents. There is broad literature to guide one in configuring appropriate expression systems for gene transfer into AVCs. This includes integrating vectors (for example, retroviral, adenoviral, adeno-associated viral, and naked DNA vectors), episomal (extrachromasomally-replicating) vectors (for example, Epstein-Barr virus and BK virus vectors), and cytoplasmic expression vectors (for example, T7 promoter/polymerase vectors). Though ex vivo gene transfer, followed by administration of the engineered AVCs to a patient, is a preferred therapeutic application of the present technology, one can readily envision in vivo gene transfer applications with cell-specific targeting vectors.

AVC engineering can also be accomplished by protein transfer, that is, the external application of a cell surface-associating or soluble protein. In the case of cell surface-associating proteins, artificially lipid-modified variants of polypeptides are preferred. Lipid

modification can be accomplished by covalently conjugating lipids to soluble derivatives of the inhibitory protein of interest. An example of a lipid modification is palmitoylation. An advantage of this approach is that soluble recombinant proteins can be readily produced in large quantities using yeast or bacterial expression systems.

Alternatively, artificial glycosyl-phosphatidylinositol ("GPI")-modified derivatives of proteins can be produced by chimeric gene transfer. GPI-modified proteins are a class of native cell surface molecules that can be exogenously reincorporated back into cell membranes after purification (Medof et al., 160 J. Exp. Med. 1558, 1984; Moran et al., 149 J. Immunol. 1736, 1992; Zhang et al., 89 Proc. Natl. Acad. Sci. USA 5231, 1992; Bulow et al., 27 Biochemistry 2384, 1988; Hitsumoto et al., 5 Int. Immunol. 805, 1993). This property stems from their amphiphilic properties and their solubility in exceedingly low detergent concentration or in the complete absence of detergent. Protein transfer has been reported for a limited set of natural GPI-anchored proteins, including decay-accelerating factor ("DAF") (Medof et al., 160 J. Exp. Med. 1558, 1984; Moran et al., 149 J. Immunol. 1736, 1992), Thy-1 (Zhang et al., 89 Proc. Natl. Acad. Sci. USA 5231, 1992), *T. brucei* variant surface glycoprotein (Bulow et al., 27 Biochemistry 2384, 1988), and mouse heat-stable antigen (Hitsumoto et al., 5 Int. Immunol. 805, 1993). For DAF and heat stable antigen, biological functions have been demonstrated for the exogenously reincorporated proteins (Moran et al., 149 J. Immunol. 1736, 1992; Zhang et al., 89 Proc. Natl. Acad. Sci. USA 5231, 1992; Hitsumoto et al., 5 Int. Immunol. 805, 1993).

Polypeptide sequences can be artificially GPI-modified by linking their coding sequences to a GPI modification signal sequence (Tykocinski et al., 85 Proc.

Natl. Acad. Sci. USA 3555, 1988; Straus et al., 82 Proc. Natl. Acad. Sci. USA 6245, 1985; Caras et al., 238 Science 1280, 1987; Waneck et al., 85 Proc. Natl. Acad. Sci. USA 577, 1988). This finding has been substantiated by other more recent studies for a variety of target proteins (Lin et al., 249 Science 677, 1990; Sleckman et al., 147 J. Immunol. 428, 1991; Zhang et al., 115 J. Cell Biol. 75, 1991; Mann et al., 142 J. Immunol. 318, 1989; Matsui et al., 254 Science 1788, 1991; Wettstein et al., 174 J. Exp. Med. 219, 1991; Kemble et al., 122 J. Cell Biol. 1253, 1993; Weber et al., 210 Exp. Cell Res. 107, 1994; Huang et al., 31 Mol. Immunol. 1017, 1994; Scheirle et al., 149 J. Immunol. 1994, 1992). Gene transfer of GPI-modified MHC is disclosed by Mann et al., 142 J. Immunol. 318, 1989; Matsui et al., 254 Science 1788, 1991; Wettstein et al., 174 J. Exp. Med. 219, 1991; Scheirle et al., 149 J. Immunol. 1994, 1992; and Huang et al., 31 Mol. Immunol. 1017, 1994.

The coding sequence for the reagent of interest, for example, the extracellular domain of the Fas-L or single chain anti-class I Fv, can be linked in-frame to a GPI modification signal sequence from the carboxy terminus of a naturally GPI-modified protein such as human decay-accelerating factor. Such artificial GPI-modified proteins can be produced in large scale using mammalian (for example, glutamine synthetase amplification/expression system) or yeast (for example, Pichia expression system) over-expression systems. The artificial GPI-modified protein can then be purified by immunoaffinity chromatography or other standard biochemical purification methods. By incorporating a polyhistidine tag into the chimeric polypeptide, in between the two distinct coding sequences, one can simplify purification through the use of nickel-sepharose chromatography. Artificial GPI-modified inhibitors can be painted onto AVC surfaces by simply combining them with

the cells, optimally for one hour at room temperature or 37°C.

In the case of soluble reagent, protein transfer can be accomplished through the use of liposomes. Methods are well described for designing liposomes to be used as polypeptide carriers, and these can be directly applied to the delivery of immunoregulatory soluble molecule to AVCs. Alternatively, inert carriers, for example, polysaccharide beads, can be pre-coated with soluble molecules and delivered to the cytoplasm of cells. Additionally, APCs can be induced to pinocytose high concentrations of soluble molecules. In all of these cases, the immunoregulatory molecule is released by the APC slowly over time.

In another aspect, the invention features soluble inhibitory reagents that have been genetically engineered to confer special properties to them. One useful modification is to combine the sequences for more than one reagent into a single polypeptide. This provides for cooperative inhibitory functions without the need for using multiple reagents. In designing such "multi-functional reagents", one can draw upon published experience with chimeric hematopoietic cytokines which have been successfully used to modulate hematopoiesis. Another useful modification is lipid modifying reagents such as cytokines that normally exist as soluble forms in order to allow them to be anchored into the APC surface. Certain cytokines are known to function in both soluble and cell surface-associated modes. According to the present invention, immunosuppressive cytokines such IL-10 and TGFβ are produced in a lipid-modified, for example, GPI-modified, form and then coated onto APC surfaces to generate AVCs. Such tethered immunoregulatory cytokines retain the inhibitory function of their natural soluble counterparts.

In another aspect the invention features cells

other than conventional APCs that are engineered to function as AVCs. This involves cells that cannot themselves efficiently process complex polypeptide antigens. In such cells, antigen presentation is accomplished by either using endogenous MHC molecules which can be loaded (or pulsed) with exogenous antigenic peptides, or by transfecting the cells with MHC genes and then loading (or pulsing) the expressed proteins, or by coating the cells with pre-formed MHC:antigenic peptide complexes.

One example of a non-conventional APC that can be converted into an AVC is a grafted cell. Cells of a graft, such as epithelial cells, can be coated with an inhibitory molecule prior to transplantation into the recipient. This represents a method for promoting engraftment, since cytotoxic T-cell effectors can be ablated.

Other clinical applications for AVCs include a diverse set of autoimmune, alloimmune, and other acute and chronic inflammatory diseases. It is well-established in the scientific literature that pathogenic T-cells play critical roles in such diseases. AVCs can be administered to such patients, for example, intravenously, subcutaneously, intramuscularly, or intraperitoneally to inhibit such pathogenic T-cells. In the case of a patient suffering from an autoimmune disease, the patient's own APCs can be obtained, for example, by purifying them from phlebotomized blood via standard Ficoll-hypaque centrifugation and subsequent isolation methods. The cells are then converted into AVCs via inhibitory expression, pulsed with a source of pathogenic antigenic peptides, and then administered back to the patient. In the case of transplantation, the patient can be treated in a similar fashion with allogeneic AVCs prepared from the graft donor's blood. In preventing graft-versus-host disease, the transplanted marrow can be pre-treated with AVCs ex vivo.

In a first aspect the invention features a method for producing an artificial veto cell capable of specifically inhibiting the proliferation, cytotoxicity or cytokine release of a T-cell or inducing apoptosis or necrotic cell death in a T-cell directed to an alloantigen or processed antigen. The method entails providing an antigen presenting cell having a specific alloantigen or processed antigen. Then the extracellular surface of the antigen presenting cell is externally contacted with a chimera comprising a non-CD8 polypeptide capable of reducing T-cell proliferation, cytotoxicity, or cytokine secretion or inducing T-cell apoptosis or necrotic cell death and a moiety sufficient to bind the chimera to the surface of the antigen presenting cell in a manner which presents the polypeptide on the cell's surface such that the polypeptide is able to reduce T-cell proliferation, cytoxicity or cytokine secretion or induce T-cell apoptosis or necrotic cell death.

In an alternative embodiment the method entails inserting a genetic sequence encoding a non-CD8 cell surface polypeptide or a chimera into the antigen presenting cell.

Another embodiment of the method features inserting into an antigen presenting cell a genetic sequence encoding a secreted molecule that is capable of reducing T-cell proliferation, cytotoxicity or cytokine secretion or inducing apoptosis or necrotic cell death in a T-cell.

In a further embodiment a secreted molecule is delivered to the antigen presenting cell in a form that allows for subsequent localized release.

By "artificial veto cell" is meant a non-naturally occurring cell that is capable of inhibiting the proliferation of, cytotoxicity of, or cytokine release by a T-cell or of inducing apoptosis or necrotic cell death in a T-cell. The cell bears an antigenic peptide at its

cell surface that can bind to the T-cell receptor of a T-cell that is being inhibited or induced to apoptose or undergo necrotic cell death. This antigenic peptide permits targeting of the inhibitory effect to antigen-specific T-cells. Generally, the cell is a molecularly modified conventional antigen-presenting cell, such as a dendritic cell or a B-cell. However, a non-antigen processing cell can also be converted to an artificial veto cell by providing an antigenic peptide to its cell surface.

By "proliferation" is meant cell division which increases the number of cells present. Cell division is associated with DNA synthesis and can be monitored ex vivo by measuring ^{3}H-thymidine incorporation.

By "cytotoxicity" is meant the capacity of one cell to kill another cell. This cell function can be monitored ex vivo by measuring ^{51}Cr release from radio-labeled target cells.

By "cytokine secretion" is meant the release from the cell of a protein that mediates an immune response.

By "apoptosis" is meant programmed cell death in response to a variety of different triggers.

By "necrotic cell death" is meant cell death mediated by an environment made so hostile to the cell, by means of poisons or inappropriate pH or lack of oxygen, that the cell cannot maintain a state of homeostasis.

By "alloantigen" is meant either class I or class II MHC molecules with or without associated antigenic peptides from a different individual of the same species.

By "processed antigen" is meant fragments of a foreign substance, usually a protein, that bind to class I or class II MHC molecules.

An antigen presenting cell can be isolated in a number of ways. They may be obtained from peripheral blood. The blood is fractionated by density gradients to obtain either the mononuclear cells or the polynuclear

cells. This procedure is well known to persons skilled in the art. Various techniques can be utilized to isolate APCs (B lymphocytes, dendritic cells, or monocytes) such as adherence, adherence and release, fluorescence activated cell sorting with lineage specific antibodies, magnetic cell sorting with lineage specific antibodies, complement mediated killing with lineage specific antibodies. Polymorphonuclear blood cells can also be used as APCs. Various tissues also contain cells that can act as APCs. These tissues can be dissociated by physical and enzymatic means to release APCs. For example, endothelial cells can be obtained from vessels and myoblasts from muscle. The ability of some of these cells to act as APCs can be enhanced by treatment of the cells with cytokines such as interferon gamma. In the case of an alloantigen, isolation of the APC from a particular source will determine the type of alloantigen presented by the APC. A specific processed antigen can be expressed on an isolated APC by either feeding unprocessed antigenic peptide to the APC and allowing the APC to process the antigenic peptide or exposing the APC to processed antigen. Those in the art are familiar with both these techniques. In this manner, an antigen presenting cell having an alloantigen or processed antigen can be provided.

By "externally contacting the extracellular surface" is meant contacting the cell surface from the exterior as opposed to insertion into a cell membrane via an intracellular route.

By "chimera" is meant a polypeptide that possesses at least one domain from one protein and at least another domain from a different protein.

By "non-CD8 polypeptide" is meant a protein other than CD8, that is involved in immunoregulation by inhibition of cytokine release, proliferation or cytotoxicity or by inducing apoptosis or necrotic cell

death. Such molecules include: secreted molecules and cell surface associated molecules.

By "moiety sufficient to bind said chimera to said surface" is meant a molecule or domain of a molecule that allows for attachment to or incorporation into the cell membrane. For example, such a molecule may be a lipid.

By "inserting into said antigen presenting cell a genetic sequence" is meant any procedure that allows for introduction of nucleic acids into a cell, e.g., transfection, electroporation, liposome transfer.

By "genetic sequence encoding a non-CD8 cell surface polypeptide" is meant the sequence of nucleic acids specific for the non-CD8 cell surface polypeptide in the context of other regulatory sequences that enable the polypeptide sequence to be transcribed into mRNA and then translated into an active protein.

By "secreted molecule" is meant a molecule that can be released by an artificial veto cell so that it is free from the antigen presenting cell.

By "genetic sequence encoding a secreted molecule" is meant the sequence of nucleic acids specific for the secreted molecule in the context of other regulatory sequences that enable the molecules sequence to be transcribed into mRNA and then translated into an active protein.

By "locally released from said antigen presenting cell" is meant secreted in a soluble form in the extracellular space around the antigen presenting cell.

By "delivering a secreted molecule" is meant providing a secreted molecule in a form that allows for its uptake by an APC and subsequent local release.

In a preferred embodiment the non-CD8 polypeptide is selected from the group consisting of polypeptides that comprise amino acid sequences from Fas ligand, an anti-class I MHC heavy chain antibody, an anti-class I MHC

heavy chain Fv chimeric polypeptide, an anti-β_2 microglobulin antibody, an anti-β_2 microglobulin Fv chimeric polypeptide, an anti-Fas antigen antibody, an anti-Fas-antigen Fv chimeric polypeptide, interleukin-10, transforming growth factor β, heterodimeric placental protein 14, homodimeric placental protein 14, and an immunoregulatory viral protein.

"Fas ligand" is a membrane bound polypeptide that is known to bind to a T-cell surface molecule Fas antigen and via this binding event induce apoptosis in said T-cell. (Brunner et al., 373 Nature 441, 1995; Dhein et al., 373 Nature 438, 1995; Ju et al., 373 Nature 444, 1995). The polypeptide to be used for AVC engineering comprises either the entire Fas ligand (amino acid residues 1-281) or the extracellular portion of Fas ligand (amino acid residues 103-281). (Takahashi et al. 6 Int. Immunol. 1567, 1994), incorporated herein by reference.

Fas antigen is defined as CD95, a cell surface molecule that binds Fas ligand.

By "anti-class I MHC heavy chain Ab" is meant an antibody molecule with specificity for the heavy polypeptide chain of a class I MHC heterodimer. Examples of anti-class I MHC antibodies that are capable of inhibiting a T-cell are 25.99, W6/32, CR1-S63, CR10-215, CR11-115, CR11-351, 5H7, Q6/64, Q1/28, 6/31, CR1, 01.65, and B1-23-2 (Tanabe, et al., 148 J. Immunol. 3202, 1992; De Felice, et al., 122 Cell. Immunol. 164, 1989; Smith, et al., 153 J. Immunol. 1054, 1994; Akiyama, et al., 91 Cell. Immunol. 477, 1985; Cavallini, et al., 154 Biochem. Biophys. Res. Commun. 723, 1988; Sterkers, et al., 131 J. Immunol. 2735, 1983). It is straightforward to clone the cDNAs corresponding to the immunoglobulin heavy and light chains from any one of the hybridomas expressing the respective anti-class I MHC heavy chain antibodies. These cDNAs can then be subcloned into expression vectors, and the resulting expression vectors can be used to express

the antibodies in cells being converted to artificial veto cells. For a membrane-associating variant, a membrane anchoring moiety can be appended to the carboxy-terminus. This can consist of a linker peptide, a hydrophobic transmembrane peptide and cytoplasmic extension (for example, an amino acid sequence corresponding to the hydrophobic transmembrane peptide and cytoplasmic extension of membrane IgG) or of a GPI modification signal sequence from a polypeptide that is naturally GPI-anchored to the membrane (for example, human decay-accelerating factor, spanning an amino acid sequence comprising Pro311 to Thr343 of this protein). Gene chimerization is readily carried out using splice-by-overlap-extension polymerase chain reaction ("SOE-PCR") technology. (Horton et al., 8 Biotechniques 528, 1990; Horton et al., 217 Methods Enzymol. 270, 1993).

By "anti-class I MHC heavy chain Fv chimeric polypeptide" is meant a recombinant fusion polypeptide comprising a leader peptide, the variable domain of the immunoglobulin heavy chain of an anti-class I MHC heavy chain Ab, a linker peptide (for example, $[(Gly)_3Ser]_3$), and the variable domain of the immunoglobulin light chain of an anti-class I MHC heavy chain Ab. For a membrane-associating variant, a membrane anchoring moiety is appended to the carboxy-terminus. This can consist of a linker peptide, a hydrophobic transmembrane peptide and cytoplasmic extension (for example, an amino acid sequence corresponding to the hydrophobic transmembrane peptide and cytoplasmic extension of membrane IgG) or of a GPI modification signal sequence from a polypeptide that is naturally GPI-anchored to the membrane (for example, human decay-accelerating factor, spanning an amino acid sequence comprising Pro311 to Thr348 of this protein). For a soluble Fv, a stop codon is appended immediately following the light chain variable domain.

By "anti-β_2 microglobulin antibody" is meant an

antibody molecule with specificity for the β_2 microglobulin chain of a class I MHC heterodimer. An example of an anti-β_2 microglobulin antibody that is capable of inhibiting a T-cell is described in Bach et al., 182 Science 1350, 1973. The strategy for cloning and expressing such an antibody is as described above for an anti-class I MHC heavy chain antibody.

By "anti-β_2 microglobulin Fv chimeric polypeptide" is meant a recombinant fusion polypeptide comprising a leader peptide, the variable domain of the immunoglobulin heavy chain of an anti-β_2 microglobulin Ab, a linker peptide (for example, $[(Gly)_3Ser]_3$), and the variable domain of the immunoglobulin light chain of an anti-β_2 microglobulin Ab. For a membrane-associating variant, a membrane anchoring moiety is appended to the carboxy-terminus. This can consist of a linker peptide, a hydrophobic transmembrane peptide and cytoplasmic extension (for example, an amino acid sequence corresponding to the hydrophobic transmembrane peptide and cytoplasmic extension of membrane IgG) or of a GPI modification signal sequence from a polypeptide that is naturally GPI-anchored to the membrane (for example, human decay-accelerating factor, spanning an amino acid sequence comprising Pro311 to Thr348 of this protein). For a soluble Fv, a stop codon is appended immediately following the light chain variable domain.

By "interleukin-10" is meant a soluble cytokine known by that designation with well-described T-cell inhibitory function. The complete amino acid sequence of this cytokine, or functional derivatives thereof, are used according to the present invention for AVC engineering. (Vieira et al., 88 Proc. Natl. Acad. Sci., USA 1172, 1991), incorporated herein by reference.

By "transforming growth factor β" is meant a soluble cytokine with described T-cell inhibitory function. The complete amino acid sequence of this

cytokine, or functional derivatives thereof, are used according to the present invention for AVC engineering. (Derynck et al., 316 Nature 701, 1985, incorporated herein by reference; Wahl et al., 140 J. Immunol. 3026, 1988).

By "heterodimeric placental protein 14" is meant a soluble cytokine with described T-cell inhibitory function. cDNAs corresponding to the PP14.1 and PP14.2 chains of the heterodimer have previously been cloned from cells of the platelet lineage (Morrow, et al., 145 Amer. J. Pathol. 1485, 1994, incorporated herein by reference). The complete amino acid sequence of this cytokine, or functional derivatives thereof, are used according to the present invention for AVC engineering.

By "homodimeric placental protein 14" is meant a soluble cytokine with described T-cell inhibitory function. cDNA corresponding to this protein has previously been cloned from pregnant endometrium. The complete amino acid sequence of this cytokine, or functional derivatives thereof, are used according to the present invention for AVC engineering.

Soluble molecules such as interleukin-10, transforming growth factor β, heterodimeric placental protein 14 and homodimeric placental protein 14, and the extracellular domain of the Fas ligand can modified into membrane associated variants by the above described methods.

By "an immunoregulatory viral protein" is meant a viral-encoded polypeptide with immunoregulatory characteristics. A viral protein shown to regulate or modify the immune response of T cells includes the native complete sequence or any partial sequence that maintains the immunoregulatory function. Examples of proteins with immunoregulatory characteristics are those of the immunodeficiency virus, HIV. Membrane associated variants can be constructed by the above described methods.

In a further preferred embodiment the secreted

molecule is interleukin-10, transforming growth factor β, an anti-class I MHC heavy chain antibody, an anti-class I MHC heavy chain Fv chimeric polypeptide, an anti-β_2 microglobulin antibody, and anti-β_2 microglobulin Fv chimeric polypeptide, an anti-Fas antigen antibody, an anti-Fas-antigen Fv chimeric polypeptide, a chimeric bacterial toxin molecule, HIV-tat, CTLA4Ig, a viral immunoregulatory protein, heterodimeric placental protein 14, homodimeric placental protein 14, or an antibody against a T-cell activation antigen.

By "HIV tat" is meant a soluble protein known by that designation and encoded by human immunodeficiency virus with well-described T-cell inhibitory function. An active domain of HIV tat consists of amino acids 1-72. (Viscidi, et al., 246 Science 1606, 1989, incorporated herein by reference). The complete amino acid sequence of this protein, or functional derivatives thereof, are used according to the present invention for AVC engineering.

By "any activation antigen of a T-cell" is meant cell surface molecules that are not expressed on T cells not stimulated by antigens but are expressed on T cells stimulated by antigens. Such molecules include: CD25, CD38, CD30, CD49a, CD49b, CD49c, CD49d, CD26, CD69, CD70, CD71, CD96, CD98, CD109, and class II MHC molecules. Antibodies to an activation antigen of a T-cell can be whole molecules or Fv. Antibodies to T-cell activation antigens can be obtained by immunizing mice with activated T lymphocytes and producing cloned hybridomas secreting antibodies by utilizing standard methods known to those skilled in the art. These monoclonal antibodies can be selected for defined specificities by comparison with the known antibodies. Alternatively, purified activation antigens or synthetic peptides produced from their determined sequences can be used to immunize mice for the production of monoclonal antibodies.

By "CTLA4Ig" is meant a soluble molecule that

inhibits the ability of CD28 or CTLA4 to ligate to its receptors on APCs which are positive for B7-1 or B7-2 (Lenschow et al., 257 Science 789, 1992; Linsley et al., 257 Science 792, 1992).

By "chimeric bacterial toxin molecule" is meant cytokines (e.g.,IL-1, Il-2, IL-4, IL-6, IL-7, TGFβ, tumor necrosis factor), antibodies (whole molecules or Fv) to activation antigens, and anti-Fas antigen molecules that are chimeric with bacterial toxins especially those from Pseudomonas (Kozak et al., 145 J. Immunol. 2766, 1990; Kreitman et al., 87 Proc. Natl. Acad. Sci., USA 8291, 1990; Lorberboum-Galski et al. 265 J. Biol. Chem. 16311, 1990; Lorberboum-Galski et al., 86 Proc. Natl. Acad. Sci., USA 1008, 1989; Lorberboum-Galski et al., 85 Proc. Natl. Acad. Sci., USA 1922, 1988, incorporated herein by reference; Ogata et al., 141 J. Immunol. 4224, 1988; Roberge et al, 143 J. Immunol. 3498, 1989) or diphtheria (Bacha et al., 22 Eur. J. Immunol. 1673, 1992; Bacha et al., 167 J. Exp. Med. 612, 1988; Bastos et al., 145 J. Immunol. 3535, 1990; Kelley et al., 85 Proc. Natl. Acad. Sci., USA 3980, 1988; Murphy et al., 83 Proc. Natl. Acad. Sci., USA 8258, 1986, incorporated herein by reference; Williams et al., 265 J. Biol. Chem. 11885, 1990; Williams et al., 265 J. Biol. Chem. 20673, 1990; Williams et al., 1 Prot. Eng. 493, 1987, incorporated herein by reference). For example, a complete cytokine sequence (e.g., IL-2) can be recombined with a bacterial toxin sequence truncated so that its cellular recognition domain is not included in the chimeric protein. The cytokine domain will act as a cellular recognition domain and the bacterial toxin sequence will provide cell destructive capabilities. Chimeric molecules are transferred to AVCs via gene transfer and are subsequently secreted from the APC.

In further preferred embodiments the antigen presented by the antigen presenting cell is bound to a class I MHC polypeptide; the antigen is bound to a class

II MHC polypeptide; the moiety comprises a lipid modification; the lipid modification comprises glycosyl-phosphatidylinositol; the GPI-modified peptide is produced by gene transfer of a chimeric gene expression construct comprising a GPI modification signal sequence into a host cell and isolation of the GPI-modified polypeptide from the host cell; the soluble molecule is delivered via a liposome; the soluble molecule is delivered via an inert bead; the soluble molecule is delivered via pinocytosis.

By "class I MHC polypeptide sequence" is meant an amino acid sequence corresponding to a portion of the extracellular domain of a class I major histocompatibility complex heavy chain, for example, an HLA-A, HLA-B, or HLA-C heavy chain.

By "class II MHC polypeptide sequence " is meant an amino acid sequence corresponding to a portion of the extracellular domain of either a class II major histocompatibility complex alpha chain or a class II major histocompatibility complex beta chain, for example, an HLA-DQ, HLA-DR, or HLA-DP alpha or beta chain.

By "lipid modification" is meant the attachment of a lipid moiety to a protein by chemical or enzymatic means.

By "glycosyl-phosphatidylinositol-modified polypeptide is meant an polypeptide which has a covalently attached glycosyl-phosphatidylinositol molecule, so as to allow membrane insertion.

By "gene transfer of a chimeric gene expression construct comprising a glycosyl-phosphatidylinositol modification signal sequence" is meant the introduction into a cell of a nucleic acid sequence encoding a chimeric gene including one domain with the nucleic acid sequence of a glycosyl-phosphatidylinositol modification signal sequence in the context of other regulatory sequences that enable the encoded sequence to be transcribed into mRNA and translated into protein and modified with a glycosyl-

phosphatidylinositol anchor.

"Isolation of the glycosyl-phosphatidylinositol polypeptide" is accomplished by chromatography techniques such as: affinity, ion exchange, hydrophobic interaction, or gel filtration. All these procedures are known to those who practice the art.

By "liposome" is meant a lipid encapsulated vessel for delivery of molecules to a cell.

By "inert bead" is meant a substance that is chemically unreactive, physically stable, and is not a substrate for an enzyme, for example a polysaccharide bead.

By "pinocytosis" is meant the process of inclusion of an extracellular substance in a cellular vesicle located in the cytosol and made from an invagination of the cell surface membrane. The use of pinocytosis to cause cells to uptake molecules is known to those who practice the art.

In other preferred embodiments the chimeric bacterial toxin comprises a cytokine that binds to a receptor on a T cell, an antibody that binds to an antigen on a T cell or a Fv molecule that binds to an antigen on a T cell; the chimeric bacterial toxin comprises the toxin domain from diphtheria toxin or Pseudomonas exotoxin.

By "cytokine that binds a receptor on a T cell" are cytokines such as IL-1, Il-2, IL-4, IL-6, IL-7, TGFβ, or tumor necrosis factor.

By "antibody that binds to an antigen on a T cell" is meant an antibody directed toward activation antigens, or anti-Fas antigen molecules.

By "Fv molecule that binds to an antigen on a T cell" is meant an Fv molecule directed toward activation antigens or anti-Fas antigen molecules.

By "toxin domain" is meant a portion of a toxin that possesses toxic activity.

By "diphtheria toxin" is meant the soluble toxin

protein that is released by the bacterium Corynebacterium diphtheria.

By "Pseudomonas exotoxin" is meant the soluble toxic protein released by Pseudomonas bacterium.

In a second aspect, the invention features a method for producing a non-naturally occurring biological membrane capable of specifically inhibiting the proliferation, cytotoxicity or cytokine secretion or inducing apoptosis or necrotic cell death in the T-cell directed to an alloantigen or processed antigen. The method comprises isolating a biological membrane having an alloantigen or processed antigen, and contacting the extracellular portion of the biological membrane with a chimera comprising a non-CD8 polypeptide capable of reducing T-cell proliferation, cytotoxicity or cytokine secretion or inducing T-cell apoptosis or necrotic cell death and a moiety sufficient to bind the chimera to the biological membrane in a manner which presents the polypeptide on the biological membrane such that the polypeptide is able to reduce T-cell proliferation, cytoxicity, or cytokine secretion or induce T-cell apoptosis or necrotic cell death.

By "non-naturally occurring biological membrane" is meant a membrane that does not naturally have the specified T cell immunoregulatory capacity, but has been provided with such capacity.

By "isolating a biological membrane having said alloantigen or processed antigen" is meant a procedure for obtaining a biological surface membrane from a cell expressing an alloantigen or processed antigen. These procedures are well known to those skilled in the art. A cell presenting an alloantigen or processed antigen is used to isolate such a biological membrane. Biological membranes are isolated by disrupting the cell through a variety of means such as freeze-thawing, nitrogen cavation, detergent lysis, or other techniques known to

those in the art. Nuclei are sedimented and discarded leaving cytosol and cell membranes. The membranes are isolated by appropriate density gradient and centrifugation techniques. For example, fractions with plasma (surface) membrane are found in specific fractions of a density gradient and they are identified with specific enzymatic activities known to characterize the surface membranes. Once the biological membrane is isolated the non-CD8 polypeptide would be bound to the membrane utilizing the same procedures as described for intact cells.

In an alternative embodiment the method encompasses providing an antigen presenting cell having a specified alloantigen or processed antigen, inserting into the antigen presenting cell a genetic sequence encoding a chimera comprising a non-CD8 polypeptide capable of reducing T-cell proliferation, cytotoxicity or cytokine secretion or inducing T-cell apoptosis or necrotic cell death and a moiety sufficient so that the chimera is expressed on the surface of the antigen presenting cell in a manner which presents the polypeptide so as to be able to reduce T-cell proliferation, cytoxicity or cytokine secretion or induce T-cell apoptosis or necrotic cell death, and isolating the biological membrane from the antigen presenting cell.

In a third aspect, the invention features a glycosyl-phosphatidylinositol-modified polypeptide comprising a polypeptide selected from the group consisting of polypeptides that comprise amino acid sequences from the extracellular domain of Fas ligand, an anti-class I MHC heavy chain antibody, an anti-class I MHC heavy chain Fv chimeric polypeptide, an anti-β_2 microglobulin antibody, and anti-β_2 microglobulin Fv chimeric polypeptide, an anti-Fas antigen antibody, an anti-Fas antigen Fv chimeric polypeptide, interleukin-10, transforming growth factor β, heterodimeric placental

protein 14, homodimeric placental protein 14, HIV tat, and an immunoregulatory viral protein, which is separate from a membrane.

In a fourth aspect, the invention features an artificial veto cell having a membrane exogenously coated with a lipid-modified non-CD8 polypeptide selected from the group consisting of polypeptides that comprise amino acid sequences from the extracellular domain of the Fas ligand, an anti-class I MHC heavy chain antibody, an anti-class I MHC heavy chain Fv chimeric polypeptide, an anti-β_2 microglobulin antibody, and anti-β_2 microglobulin Fv chimeric polypeptide, an anti-Fas antigen antibody, an anti-Fas antigen Fv chimeric polypeptide, interleukin-10, transforming growth factor β, heterodimeric placental protein 14, homodimeric placental protein 14, and an immunoregulatory viral protein.

In a preferred embodiment the artificial veto cell comprises a genetic sequence that encodes for the lipid-modified non-CD8 polypeptide.

By "genetic sequence that encodes a cell surface lipid-modified non-CD8 polypeptide" is meant a nucleic acid sequence encoding a polypeptide including a lipid modification sequence and other regulatory sequences that enable the encoded sequence to be transcribed into mRNA, translated into protein and modified with a lipid moiety.

In a fifth aspect the invention features an artificial veto cell bearing a transfected genetic sequence that encodes a secreted molecule capable of reducing T-cell proliferation, cytotoxicity, or cytokine secretion or inducing apoptosis or necrotic cell death directed to alloantigens or processed antigens.

By "bearing a transfected genetic sequence" is meant a genetic sequence encoding for a secreted molecule either integrated into a chromosome or existing extrachromosomally.

In a sixth aspect the invention features an

artificial veto cell containing an exogenously added secreted molecule capable of reducing T-cell proliferation or cytotoxicity, or cytokine secretion or inducing apoptosis or necrotic cell death directed to alloantigens or processed antigens. The artificial antigen presenting cell contains the exogenously added secreted molecule by carrier-delivery, pinocytosis, or liposome delivery.

In a preferred embodiment the secreted molecule is selected from the group consisting of interleukin-10, transforming growth factor β, an anti-class I MHC heavy chain antibody, an anti-class I MHC heavy chain Fv chimeric polypeptide, an anti-β_2 microglobulin antibody, an anti-β_2 microglobulin Fv chimeric polypeptide, an anti-Fas antigen antibody, an anti-Fas-antigen Fv chimeric polypeptide, a chimeric bacterial toxin molecule, HIV-tat, CTLA4Ig, a viral immunoregulatory protein, heterodimeric placental protein 14, homodimeric placental protein 14, and an antibody against a T-cell activation antigen.

In a seventh aspect, the invention features a method for inhibiting an antigen-specific T-cell. The method comprises providing an artificial veto cell which presents in, or on its surface a non-CD8 polypeptide capable of reducing T-cell proliferation, cytotoxicity or cytokine secretion or inducing T-cell apoptosis or necrotic cell death and an MHC:nominal antigen peptide complex, and exposing the T-cell capable of responding to the MHC:nominal antigen peptide complex so as to inhibit the cellular immune response of the T cells to the antigen.

By "MHC:nominal antigen peptide complex is meant a class I or class II MHC molecule with a noncovalent or covalently associated antigenic peptide.

By "exposing" is meant brought into proximity so as to allow molecules either bound to or released from the antigen presenting cell to contact the T cell.

In a preferred embodiment the non-CD8 polypeptide

is selected from the group consisting of polypeptides that comprise amino acid sequences from Fas ligand, an anti-class I MHC heavy chain antibody, an anti-class I MHC heavy chain Fv chimeric polypeptide, an anti-β_2 microglobulin antibody, and anti-β_2 microglobulin Fv chimeric polypeptide, an anti-Fas antigen antibody, an anti-Fas antigen Fv chimeric polypeptide, interleukin-10, transforming growth factor β, heterodimeric placental protein 14, homodimeric placental protein 14, HIV tat, and an immunoregulatory viral protein.

In an eighth aspect the invention features a method for inhibiting an antigen-specific T-cell by providing an artificial veto cell which locally releases a secreted molecule and an MHC:nominal antigen peptide complex present on its surface, and exposing the T-cells capable of responding to the MHC:nominal antigen peptide complex so that the secreted molecule is locally released and inhibits the cellular immune response of the T cells to the antigen.

In a preferred embodiment the secreted molecule is selected from the group consisting of interleukin-10, transforming growth factor β, an anti-class I MHC heavy chain antibody, an anti-class I MHC heavy chain Fv chimeric polypeptide, an anti-β_2 microglobulin antibody, an anti-β_2 microglobulin Fv chimeric polypeptide, an anti-Fas antigen antibody, an anti-Fas-antigen Fv chimeric polypeptide, a chimeric bacterial toxin molecule, HIV-tat, CTLA4Ig, a viral immunoregulatory protein, heterodimeric placental protein 14, homodimeric placental protein 14, and an antibody against a T-cell activation antigen.

In preferred embodiments T-cells are exposed ex vivo; T-cells are exposed in vivo; the MHC polypeptide is syngeneic with the antigen-specific T-cell; the MHC polypeptide is allogeneic with the antigen-specific T-cell.

By "ex vivo" is meant outside of the body of a patient to be treated.

By "in vivo" is meant in the body of a patient to be treated.

By "syngeneic" is meant that the MHC molecules of the antigen-specific T-cell is identical to that of the MHC molecule on the artificial veto cell that is being transferred.

By "allogeneic" is meant that the antigen-specific T-cell bears at least one MHC allelic variant that is disparate with that of the MHC molecule on the artificial veto cell that is being transferred.

In a tenth aspect, the invention features a method for inhibiting a pathogenic T-cell in a patient. The method comprises administering to the patient an artificial veto cell which presents in, or on its surface a polypeptide selected from the group consisting of polypeptides that comprise amino acid sequences from Fas ligand, an anti-class I MHC heavy chain antibody, an anti-class I MHC heavy chain Fv chimeric polypeptide, an anti-β_2 microglobulin antibody, and anti-β_2 microglobulin Fv chimeric polypeptide, an anti-Fas antigen antibody, an anti-Fas antigen Fv chimeric polypeptide, interleukin-10, transforming growth factor β, heterodimeric placental protein 14, homodimeric placental protein 14, and an immunoregulatory viral protein, and an MHC:nominal antigen peptide complex.

In another embodiment, the method for inhibiting a pathogenic T-cell in a patient may be practiced by utilizing an artificial veto cell which locally releases a molecule selected from the group consisting of interleukin 10, transforming growth factor β, an anti-class I MHC heavy chain antibody, an anti-class I MHC heavy chain Fv chimeric polypeptide, an anti-β_2 microglobulin antibody, an anti-β_2 microglobulin Fv chimeric polypeptide, an anti-Fas antigen antibody, an

anti-Fas-antigen Fv chimeric polypeptide, a chimeric bacterial toxin molecule, HIV-tat, CTLA4Ig, a viral immunoregulatory protein, heterodimeric placental protein 14, homodimeric placental protein 14, and an antibody against a T-cell activation antigen.

By "administering to the patient" is meant given internally to a patient.

In an eleventh aspect, the invention features various membrane associated antibodies. These include: an anti-class I MHC antibody comprising an immunoglobulin heavy chain polypeptide and an immunoglobulin light chain polypeptide, an anti-β_2 microglobulin antibody comprising an immunoglobulin heavy chain polypeptide and an immunoglobulin light chain polypeptide, an anti-Fas antigen antibody comprising an immunoglobulin heavy chain polypeptide and an immunoglobulin light chain polypeptide, an anti-class I MHC Fv chimeric polypeptide, anti-β_2 microglobulin Fv chimeric polypeptide, an anti-Fas antigen chimeric polypeptide.

In preferred embodiments the antibodies are lipid-modified; the lipid modification is a glycosyl-phosphatidylinositol moiety; the polypeptide comprises a poly-histidine tag.

By "poly-histidine tag" is meant two or more clustered histidines that are inserted into a polypeptide sequence in order to permit purification of the polypeptide by nickel-sepharose chromatography. In the case of a glycosyl-phosphatidylinositol-modified polypeptide, an optimal site for insertion of the polyhistidine amino acid sequence is in between the sequence for the polypeptide of interest and the GPI modification signal sequence. In the case of GPI-modified polypeptides, the N-terminal signal peptide and C-terminal GPI moiety preclude polyhistidine insertion into the conventional N-terminal and C-terminal sites. Insertion of the polyhistidine sequence is accomplished by inserting the coding sequence for this

polyhistidine sequence into the desired site of an expression construct.

In a twelfth aspect, the invention features a method for producing an artificial veto cell utilizing a non-antigen presenting cell. The methods are analogous to those described for antigen presenting cells. However, initially, a non-antigen presenting cell is isolated. Such a cell is manipulated so that an alloantigen or processed antigen is presented on the extracellular surface of the cell.

By "non-antigen presenting cell" is meant a cell that does not efficiently process antigenic proteins into antigenic peptides. Generally, such a cell lacks costimulator function as well.

Non-antigen presenting cells are isolated utilizing the same procedures as utilized for the isolation of antigen presenting cells.

By "manipulated so that an alloantigen or processed antigen is presented" is meant treating the cell so that it presents an antigen.

In preferred embodiments manipulating comprises loading the cells with antigenic peptides; manipulating comprises transfecting the cells with MHC genes and loading the cells with antigenic peptides; manipulating comprises coating the cells with pre-formed MHC: antigenic peptide complexes.

By "loading the cells with antigenic peptides" is meant introduction of specific antigenic peptides into association with class I or class II MHC molecules. Such loading techniques are know to those who practice the art.

By "transfecting the cells with MHC genes" is meant introducing into the cells nucleic acid sequences encoding MHC proteins in the context of other regulatory sequences that enable the encoded sequences to be transcribed into mRNA and translated into MHC proteins.

By "coating the cells with pre-formed

MHC:antigenic peptide complexes" is meant treating cells with MHC complexed with antigenic peptides such that the complex adheres to cells.

In other preferred embodiments the polypeptide is selected from the group consisting of polypeptides that comprise amino acid sequences from Fas ligand, an anti-class I MHC heavy chain antibody, an anti-class I MHC heavy chain Fv chimeric polypeptide, an anti-β_2 microglobulin antibody, and anti-β_2 microglobulin Fv chimeric polypeptide, an anti-Fas antigen antibody, an anti-Fas antigen Fv chimeric polypeptide, interleukin-10, transforming growth factor β, heterodimeric placental protein 14, homodimeric placental protein 14, and an immunoregulatory viral protein; the secreted molecule is selected from the group consisting of interleukin-10, transforming growth factor β, an anti-class I MHC heavy chain antibody, an anti-class I MHC heavy chain Fv chimeric polypeptide, an anti-β_2 microglobulin antibody, an anti-β_2 microglobulin Fv chimeric polypeptide, an anti-Fas antigen antibody, an anti-Fas-antigen Fv chimeric polypeptide, a chimeric bacterial toxin molecule, HIV-tat, CTLA4Ig, a viral immunoregulatory protein, heterodimeric placental protein 14, homodimeric placental protein 14, or an antibody against a T-cell activation antigen.

Other features and advantages of the invention will be apparent from the following description of the preferred embodiments thereof, and from the claims.

Descriptions of the Preferred Embodiments

All techniques utilized to produce the reagents of the current invention are well known to those who practice the art. Many of the cloning and protein expression, gene transfer, protein transfer, and therapeutic protocols are described in U.S. Patent No. 5,242,687, incorporated herein by reference. All these techniques are known to those who practice the art.

Chimeric coding sequences are generally assembled using the splice-by-overlap-extension polymerase chain reaction ("SOE-PCR") method (Horton, et al., 8 BioTechniques 528, 1990). This technique has been used to generate chimeric coding sequences for artificial GPI modified proteins (Huang, et al., 31 Molec. Immunol. 1017, 1994). This is readily adapted for generating GPI- or hydrophobic peptide-anchored variants of polypeptides to be used for AVC engineering. Similarly, this approach can be used for generating Fv chimeric polypeptides and to generate chimeric bacterial toxin molecules. The SOE-PCR method is rapid and is not dependent upon having convenient restriction endonuclease cleavage sites at specific regions of the native polypeptide's coding sequence. By incorporating convenient restriction endonuclease sites into the outer SOE-PCR primers, subsequently subcloning into expression vectors is greatly simplified.

The present invention encompasses a wide range of alterations of the non-CD8 polypeptides, e.g. substitutions and deletions, that keep intact the active portion of the molecule. For cell surface associated molecules the active portion of the molecule generally comprises the extracellular domain. For secreted molecules such as cytokines the capacity to bind to specific receptors is essential although not always sufficient for activity. Active domains or essential components of proteins can be determined by mutagenesis procedures well known in the art. For example, portions of the Fas ligand molecule can be truncated or mutated or otherwise modified and then tested to see if the altered molecule retains its ability to inhibit T lymphocytes. For the Fas ligand the extracellular domain includes amino acids 103 to 281 (Takahashi et al., 6 Inter. Immunol. 1567, 1994). For an IL-2-bacterial toxin chimera the deletion of the glutamine at amino acid position 74 of the

IL-2 moiety or a mutation of alanine at amino acid position 50 to tryptophan results in molecules with biological activity equivalent to the native IL-2 molecule. (Ju et al. 262 J. Biol. Chem. 5723, 1987, incorporated herein by reference). For HIV tat amino acid residues 1 to 72 have been shown to possess immunoregulatory effects. (Viscidi et al. 246 Science 1606, 1989).

Therapeutic treatment utilizing AVCs

The treatment of a patient with an inflammatory bowel disease, such as regional enteritis (Crohn's disease), includes the following steps to be performed for the inhibition or elimination of anti-bowel T cells using Fas ligand coated artificial veto cells. First, peripheral blood is obtained from the patient via phlebotomy, a mononuclear cell fraction is isolated from the blood by Ficoll-hypaque gradient centrifugation, and the B-cell population is enriched using negative immuno-magnetic bead selection. The B-cells are activated by incubating with killed Staphylococcus aureus, Cowen strain or with protein A coated sepharose beads or with a combination of anti-IgM and lipopolysaccharide according to well-established methods. Cytokines may be added to these cultures to enhance the activation process. After 3 days the activated B cells are fed colonic cell extract for 2 hours at 37°C to allow for the uptake and processing of antigen. After the cells are washed, they are incubated for an additional hour at 37°C in the absence of serum in the presence of RPMI 1640 with 25 micrograms per ml of lipid-conjugated Fas-Ligand (extracellular domain). These artificial veto cells, either untreated or irradiated or chemically treated to irreversibly prevent further growth are then introduced into the patient. Standard clinical protocols are used to establish an optimal dosing schedule.

To prevent rejection of a solid organ transplant,

such as kidney graft, the prospective transplant recipient undergoes pretransplant transfusion with donor activated B cells coated with lipidated Fas-ligand. Cell retrieval and protein transfer are performed as described above; of note, antigenic pulsing of the cells is not required in this circumstance.

Alternatively, Fas ligand cDNA in an expression vector can be transferred into the B-cells and Fas ligand expressing cells are utilized.

Artificial veto cells containing genetic sequences encoding for soluble molecules, e.g., cytokines, Fv or heavy chain antibodies or chimeric bacterial toxin molecules, or artificial veto cells to which soluble molecules have been delivered, can be utilized as described above, with or without antigenic pulsing.

Some of the other diseases that could be treated utilizing the methods and compositions of the present invention include graft versus host rejection, diabetes, pernicious anemia, autoimmune hepatitis, and allergic diseases.

In vitro use

The treatment of a bone marrow specimen in order to prevent graft versus host disease subsequent to transplantation into a recipient includes the following steps to be performed when using Fas-Ligand coated artificial veto cells. First, peripheral blood is obtained from the prospective recipient via phlebotomy, a mononuclear cell fraction is isolated from the blood by Ficoll/hypaque gradient centrifugation, and the B lymphocyte population is enriched using negative immuno-magnetic bead selection. The B cells are activated by incubating with killed Staphylococcus aureus, Cowen strain or with protein A coated sepharose beads or with a combination of anti-IgM and lipopolysaccharide according to well-established methods. Cytokines may be added to these cultures to enhance the activation process. After

3 days the activated B cells are washed and then incubated for an hour at 37°C in the absence of serum in the presence of RPMI 1640 with 25 micrograms per ml of lipid-conjugated Fas-Ligand (extracellular domain). These artificial veto cells, either irradiated or chemically treated to irreversibly prevent further growth or not treated, are then added to the bone marrow specimen and allowed to incubate overnight at 37°C in appropriate culture medium, so as to delete bone marrow T cells with specificity against the recipient.

All references mentioned herein are incorporated by reference in their totality (including drawings).

Other embodiments are within the following claims.

CLAIMS

1. A method for producing an artificial veto cell capable of specifically inhibiting the proliferation, cytotoxicity or cytokine secretion or inducing apoptosis or necrotic cell death of a T-cell directed to alloantigen or processed antigen comprising the steps of:

a) providing an antigen presenting cell having said alloantigen or processed antigen, and

b) externally contacting the extracellular surface of said antigen presenting cell with a chimera comprising a non-CD8 polypeptide capable of reducing T-cell proliferation, cytotoxicity or cytokine secretion or inducing T-cell apoptosis or necrotic cell death and a moiety sufficient to bind said chimera to said surface of said antigen presenting cell in a manner which presents said polypeptide on said cell's surface such that said polypeptide is able to reduce T-cell proliferation, cytoxicity or cytokine secretion or induce T-cell apoptosis or necrotic cell death.

2. A method for producing an artificial veto cell capable of specifically inhibiting the proliferation, cytotoxicity or cytokine secretion or inducing apoptosis or necrotic cell death of a T-cell directed to an alloantigen or processed antigen comprising the steps of:

a) providing an antigen presenting cell having said alloantigen or processed antigen, and

b) inserting into said antigen presenting cell a genetic sequence encoding a non-CD8 cell surface polypeptide capable of reducing T-cell proliferation, cytotoxicity or cytokine secretion or inducing T-cell apoptosis or necrotic cell death, such that said polypeptide is expressed on said cell's surface so as to be able to

reduce T-cell proliferation, cytoxicity or cytokine secretion or induce T-cell apoptosis or necrotic cell death.

3. A method for producing an artificial veto cell capable of specifically inhibiting the proliferation, cytotoxicity or cytokine secretion or inducing apoptosis or necrotic cell death of a T-cell directed to an alloantigen or processed antigen comprising the steps of:

a) providing an antigen presenting cell having said alloantigen or processed antigen, and

b) inserting into said antigen presenting cell a genetic sequence encoding a chimera comprising a non-CD8 polypeptide capable of reducing T-cell proliferation, cytotoxicity, or cytokine secretion or inducing T-cell apoptosis or necrotic cell death and a moiety sufficient so that said chimera is expressed on the surface of said antigen presenting cell in a manner which presents said polypeptide so as to be able to reduce T-cell proliferation or cytoxicity or induce T-cell apoptosis or necrotic cell death.

4. A method for producing an artificial veto cell capable of specifically inhibiting the proliferation, cytotoxicity, or cytokine secretion or inducing apoptosis or necrotic cell death of a T-cell directed to an alloantigen or processed antigen comprising the steps of:

a) providing an antigen presenting cell having said alloantigen or processed antigen, and

b) inserting into said antigen presenting cell a genetic sequence encoding a secreted molecule that is capable of reducing T-cell proliferation, cytotoxicity or cytokine secretion or inducing apoptosis or necrotic cell death in a T-cell, such that said secreted molecule is locally released from said antigen presenting cell.

5. A method for producing an artificial veto cell capable of specifically inhibiting the proliferation, cytotoxicity or cytokine secretion or inducing apoptosis or necrotic cell death of a T-cell directed an alloantigen or processed antigen comprising the steps of:

a) providing an antigen presenting cell having said alloantigen or processed antigen, and

b) delivering a secreted molecule that is capable of reducing T-cell proliferation, cytotoxicity or cytokine secretion or inducing apoptosis or necrotic cell death in a T-cell, to the cytoplasm of said antigen presenting cell such that said secreted molecule is locally released from said antigen presenting cell.

6. A method for producing a non-naturally occurring biological membrane capable of specifically inhibiting the proliferation, cytotoxicity, cytokine secretion or inducing apoptosis or necrotic cell death in a T-cell directed to an alloantigen or processed antigen comprising the steps of:

a) isolating a biological membrane having said alloantigen or processed antigen, and

b) contacting the extracellular portion of said biological membrane with a chimera comprising a non-CD8 polypeptide capable of reducing T-cell proliferation, cytotoxicity, or cytokine secretion or inducing T-cell apoptosis or necrotic cell death and a moiety sufficient to bind said chimera to said surface of said biological membrane in a manner which presents said polypeptide on said biological membrane surface such that said polypeptide is able to reduce T-cell proliferation, cytoxicity, cytokine secretion or induce T-cell apoptosis or necrotic cell death.

7. A method for producing a non-naturally occurring biological membrane capable of specifically inhibiting the proliferation, cytotoxicity or cytokine secretion of a T-cell or inducing apoptosis or necrotic cell death in said T-cell directed to an alloantigen or processed antigen comprising the steps of:

a) isolating an antigen presenting cell having said alloantigen or processed antigen,

b) inserting into said antigen presenting cell a genetic sequence encoding a chimera comprising a non-CD8 polypeptide capable of reducing T-cell proliferation, cytotoxicity or cytokine secretion or inducing T-cell apoptosis or necrotic cell death and a moiety sufficient so that said chimera is expressed on the surface of said antigen presenting cell in a manner which presents said polypeptide so as to be able to reduce T-cell proliferation, cytoxicity or cytokine secretion or induce T-cell apoptosis or necrotic cell death, and

c) isolating said biological membrane from said cell.

8. The method of claim 4, wherein said secreted molecule is selected from the group consisting of interleukin-10, transforming growth factor β, an anti-class I MHC heavy chain antibody, an anti-class I MHC heavy chain Fv chimeric polypeptide, an anti-β_2 microglobulin antibody, and anti-β_2 microglobulin Fv chimeric polypeptide, an anti-Fas antigen antibody, an anti-Fas-antigen Fv chimeric polypeptide, a chimeric bacterial toxin molecule, HIV-tat, CTLA4Ig, a viral immunoregulatory protein, heterodimeric placental protein 14, homodimeric placental protein 14, and an antibody against a T-cell activation antigen.

9. The method of claim 1, 2, 3, 6 or 7 wherein said polypeptide is selected from the group consisting of polypeptides that comprise amino acid sequences from Fas ligand, an anti-class I MHC heavy chain antibody, an anti-class I MHC heavy chain Fv chimeric polypeptide, an anti-β_2 microglobulin antibody, and anti-β_2 microglobulin Fv chimeric polypeptide, an anti-Fas antigen antibody, an anti-Fas-antigen Fv chimeric polypeptide, interleukin-10, transforming growth factor β, heterodimeric placental protein 14, homodimeric placental protein 14 , and a viral immunoregulatory protein.

10. The method of claim 5, wherein said secreted molecule is selected from the group consisting of interleukin-10, transforming growth factor β, an anti-class I MHC heavy chain antibody, an anti-class I MHC heavy chain Fv chimeric polypeptide, an anti-β_2 microglobulin antibody, and anti-β_2 microglobulin Fv chimeric polypeptide, an anti-Fas antigen antibody, an anti-Fas-antigen Fv chimeric polypeptide, a chimeric bacterial toxin molecule, HIV-tat, CTLA4Ig, a viral immunoregulatory protein, heterodimeric placental protein 14, homodimeric placental protein 14, and an antibody against a T-cell activation antigen.

11. The method of claim 1, 2, 3, 4, 5, 6 or 7 wherein an antigenic peptide presented by said antigen presenting cell is bound to a class I MHC polypeptide.

12. The method of claim 1, 2, 3, 4, 5, 6 or 7 wherein an antigenic peptide presented by said antigen presenting cell is bound to a class II MHC polypeptide.

13. The method of claim 1, 3, 6 or 7 wherein said moiety comprises a lipid modification.

14. The method of claim 13 wherein said lipid modification comprises glycosyl-phosphatidylinositol.

15. The method of claim 14 wherein said GPI-modified polypeptide is produced by the steps of:

a) gene transfer of a chimeric gene expression construct comprising a glycosyl-phosphatidylinositol modification signal sequence into a host cell; and

b) isolation of said GPI-modified polypeptide from said host cell.

16. The method of claim 5 wherein said secreted molecule is delivered to said antigen presenting cell via a liposome.

17. The method of claim 5 wherein said secreted molecule is delivered to said antigen presenting cell via an inert bead.

18. The method of claim 5 wherein said secreted molecule is delivered to said antigen presenting cell via pinocytosis.

19. A glycosyl-phosphatidylinositol-modified polypeptide comprising a polypeptide selected from the group consisting of polypeptides that comprise amino acid sequences from the extracellular domain of Fas ligand, an anti-class I MHC heavy chain antibody, an anti-class I MHC heavy chain Fv chimeric polypeptide, an anti-β_2 microglobulin antibody, and anti-β_2 microglobulin Fv chimeric polypeptide, an anti-Fas antigen antibody, an anti-Fas antigen Fv chimeric polypeptide, interleukin-10, transforming growth factor β, heterodimeric placental protein 14, homodimeric placental protein 14 and an immunoregulatory viral protein, which is separate from a membrane.

20. An artificial veto cell having a membrane exogenously coated with a lipid-modified non CD8 polypeptide selected from the group consisting of polypeptides that comprise amino acid sequences from extracellular domain of Fas ligand, an anti-class I MHC heavy chain antibody, an anti-class I MHC heavy chain Fv chimeric polypeptide, an anti-β_2 microglobulin antibody, and anti-β_2 microglobulin Fv chimeric polypeptide, an anti-Fas antigen antibody, an anti-Fas antigen Fv chimeric polypeptide, interleukin-10, transforming growth factor β, heterodimeric placental protein 14, homodimeric placental protein 14 and an immunoregulatory viral protein.

21. An artificial veto cell bearing a transfected genetic sequence that encodes a cell surface lipid modified non-CD8 polypeptide selected from the group consisting of polypeptides that comprise amino acid sequences from Fas ligand, an anti-class I MHC heavy chain antibody, an anti-class I MHC heavy chain Fv chimeric polypeptide, an anti-β_2 microglobulin antibody, and anti-β_2 microglobulin Fv chimeric polypeptide, an anti-Fas antigen antibody, an anti-Fas antigen Fv chimeric polypeptide, interleukin-10, transforming growth factor β, heterodimeric placental protein 14, homodimeric placental protein 14, and an immunoregulatory viral protein.

22. An artificial veto cell bearing a transfected genetic sequence that encoding a secreted molecule capable of reducing T-cell proliferation, cytotoxicity or cytokine secretion, or inducing apoptosis or necrotic cell death directed to alloantigens or processed antigens.

23. The method of claim 22, wherein said secreted molecule is selected from the group consisting of interleukin-10, transforming growth factor β, an anti-class I MHC heavy chain antibody, an anti-class I MHC

heavy chain Fv chimeric polypeptide, an anti-β_2 microglobulin antibody, and anti-β_2 microglobulin Fv chimeric polypeptide, an anti-Fas antigen antibody, an anti-Fas-antigen Fv chimeric polypeptide, a chimeric bacterial toxin molecule, HIV-tat, CTLA4Ig, a viral immunoregulatory protein, heterodimeric placental protein 14, homodimeric placental protein 14, and an antibody against a T-cell activation antigen.

24. An artificial veto cell containing an exogenously added secreted molecule capable of reducing T-cell proliferation, cytotoxicity, or cytokine secretion or inducing apoptosis or necrotic cell death directed to alloantigens or processed antigens.

25. The artificial veto cell of claim 24, wherein said secreted molecule is selected from the group consisting of interleukin-10, transforming growth factor β, an anti-class I MHC heavy chain antibody, an anti-class I MHC heavy chain Fv chimeric polypeptide, an anti-β_2 microglobulin antibody, and anti-β_2 microglobulin Fv chimeric polypeptide, an anti-Fas antigen antibody, an anti-Fas-antigen Fv chimeric polypeptide, a chimeric bacterial toxin molecule, HIV-tat, CTLA4Ig, a viral immunoregulatory protein, heterodimeric placental protein 14, homodimeric placental protein 14, and an antibody against a T-cell activation antigen.

26. A method for inhibiting an antigen-specific T-cell comprising the steps of:

a) providing an artificial veto cell which presents in, or on its surface a non-CD8 polypeptide capable of reducing T-cell proliferation, cytotoxicity or cytokine secretion or inducing T-cell apoptosis or necrotic cell death and an MHC:nominal antigen peptide complex, and

b) exposing said T-cells capable of responding to said MHC:nominal antigen peptide complex so as to inhibit the cellular immune response of said T cells to said antigen.

27. A method for inhibiting an antigen-specific T-cell comprising the steps of:

a) providing an artificial veto cell which locally releases a secreted molecule and has an MHC:nominal antigen peptide complex on its surface, and

b) exposing said T-cells capable of responding to said MHC:nominal antigen peptide complex so that the secreted molecule inhibits the cellular immune response of said T cells to said antigen.

28. The method of claim 27, wherein the secreted molecule is selected from the group consisting of interleukin-10, transforming growth factor β, an anti-class I MHC heavy chain antibody, an anti-class I MHC heavy chain Fv chimeric polypeptide, an anti-β_2 microglobulin antibody, an anti-β_2 microglobulin Fv chimeric polypeptide, an anti-Fas antigen antibody, an anti-Fas-antigen Fv chimeric polypeptide, a chimeric bacterial toxin molecule, HIV-tat, CTLA4Ig, a viral immunoregulatory protein, heterodimeric placental protein 14, homodimeric placental protein 14, or an antibody against a T-cell activation antigen.

29. The method of claim 26 or 27 wherein said exposing step is performed *ex vivo*.

30. The method of claim 26 or 27 wherein said contacting step is performed *in vivo*.

31. The method of claim 26 or 27 wherein said MHC polypeptide is syngeneic with said antigen-specific T-cell.

32. The method of claim 26 or 27 wherein said MHC polypeptide is allogeneic with said antigen-specific T-cell.

33. A method for inhibiting a pathogenic T-cell in a patient comprising the step of:

a) administering to said patient an artificial veto cell which presents in, or on its surface a polypeptide selected from the group consisting of polypeptides that comprise amino acid sequences from Fas ligand, an anti-class I MHC heavy chain antibody, an anti-class I MHC heavy chain Fv chimeric polypeptide, an anti-β_2 microglobulin antibody, and anti-β_2 microglobulin Fv chimeric polypeptide, an anti-Fas antigen antibody, an anti-Fas antigen Fv chimeric polypeptide, interleukin-10, transforming growth factor β, heterodimeric placental protein 14, homodimeric placental protein 14, and an immunoregulatory viral protein and an MHC:nominal antigen peptide complex.

34. A method for inhibiting a pathogenic T-cell in a patient comprising the step of:

a) administering to said patient an artificial veto cell which locally releases a molecule selected from the group consisting of interleukin 10, transforming growth factor β, an anti-class I MHC heavy chain antibody, an anti-class I MHC heavy chain Fv chimeric polypeptide, an anti-β_2 microglobulin antibody, an anti-β_2 microglobulin Fv chimeric polypeptide, an anti-Fas antigen antibody, an anti-Fas-antigen Fv chimeric polypeptide, a chimeric bacterial toxin molecule, HIV-tat, CTLA4Ig, a viral

immunoregulatory protein, heterodimeric placental protein 14, homodimeric placental protein 14, and an antibody against a T-cell activation antigen.

35. A membrane-associated anti-class I MHC antibody comprising an immunoglobulin heavy chain polypeptide and an immunoglobulin light chain polypeptide.

36. A membrane-associated anti-β_2 microglobulin antibody comprising an immunoglobulin heavy chain polypeptide and an immunoglobulin light chain polypeptide.

37. A membrane-associated anti-Fas antigen antibody comprising an immunoglobulin heavy chain polypeptide and an immunoglobulin light chain polypeptide.

38. A membrane-associated anti-class I MHC Fv chimeric polypeptide.

39. A membrane-associated anti-β_2 microglobulin Fv chimeric polypeptide.

40. A membrane-associated anti-Fas antigen chimeric polypeptide.

41. The polypeptide of claim 35, 36, 37, 38, 39, or 40 wherein said polypeptide is lipid-modified.

42. The lipid-modified polypeptide of claim 41 wherein said lipid modification is a glycosyl-phosphatidylinositol moiety.

43. The polypeptide of claim 35, 36, 37, 38, 39, or 40 wherein said polypeptide comprises a poly-histidine tag.

44. A method for producing an artificial veto cell capable of specifically inhibiting the proliferation, cytotoxicity, cytokine secretion or inducing apoptosis or necrotic cell death of a T-cell directed to an alloantigen or processed antigen comprising the steps of:

a) isolating a non-antigen presenting cell,

b) externally contacting the extracellular surface of said cell with a chimera comprising a non-CD8 polypeptide capable of reducing T-cell proliferation, cytotoxicity, or cytokine secretion or inducing T-cell apoptosis or necrotic cell death and a moiety sufficient to bind said chimera to said surface of said cell in a manner which presents said polypeptide on said cell's surface such that said polypeptide is able to reduce T-cell proliferation, cytoxicity, cytokine secretion or induce T-cell apoptosis or necrotic cell death, and

c) manipulating said cell so that an alloantigen or processed antigen is presented on the extracellular surface of said cell.

45. A method for producing an artificial veto cell capable of specifically inhibiting the proliferation, cytotoxicity or cytokine secretion or inducing apoptosis or necrotic cell death of a T-cell directed to alloantigens or processed antigens comprising the steps of:

a) isolating a non-antigen presenting cell,

b) inserting into said cell a genetic sequence encoding a non-CD8 cell surface polypeptide capable of reducing T-cell proliferation, cytotoxicity, or cytokine secretion or inducing T-cell apoptosis or necrotic cell death, such that said polypeptide is expressed on said cell's surface so as to be able to reduce T-cell proliferation, cytoxicity, or cytokine secretion or induce T-cell apoptosis or necrotic cell death, and

c) manipulating said cell so that an alloantigen or processed antigen is presented on the extracellular surface of said cell.

46. A method for producing an artificial veto cell capable of specifically inhibiting the proliferation, cytotoxicity, cytokine secretion or inducing apoptosis or necrotic cell death of a T-cell directed to alloantigens or processed antigens comprising the steps of:

a) isolating a non-antigen presenting cell,

b) inserting into said cell a genetic sequence encoding a chimera comprising a non-CD8 polypeptide capable of reducing T-cell proliferation, cytotoxicity or cytokine secretion or inducing T-cell apoptosis or necrotic cell death and a moiety sufficient so that said chimera is expressed on the surface of said cell in a manner which presents said polypeptide so as to be able to reduce T-cell proliferation, cytoxicity, or cytokine secretion or induce T-cell apoptosis or necrotic cell death, and

c) manipulating said cell so that an alloantigen or processed antigen is presented on the extracellular surface of said cell.

47. A method for producing an artificial veto cell capable of specifically inhibiting the proliferation, cytotoxicity, or cytokine secretion or inducing apoptosis or necrotic cell death of a T-cell directed to alloantigens or processed antigens comprising the steps of:

a) isolating a non-antigen presenting cell,

b) inserting into said cell a genetic sequence encoding a secreted molecule that is capable of reducing T-cell proliferation, cytotoxicity, or cytokine secretion or inducing apoptosis or necrotic cell death in a T-cell,

such that said secreted molecule is locally released from said cell, and

c) manipulating said cell so that an alloantigen or processed antigen is presented on the extracellular surface of said cell.

48. A method for producing an artificial veto cell capable of specifically inhibiting the proliferation, cytotoxicity, cytokine secretion or inducing apoptosis or necrotic cell death of a T-cell directed to alloantigens or processed antigens comprising the steps of:

a) isolating a non-antigen presenting cell,

b) delivering a secreted molecule that is capable of reducing T-cell proliferation, cytotoxicity, or cytokine secretion or inducing apoptosis or necrotic cell death in a T-cell, to the cytoplasm of said cell, such that said secreted molecule is locally released from said cell, and

c) manipulating said cell so that an alloantigen or processed antigen is presented on the extracellular surface of said cell.

49. The method of claims 44, 45, 46, 47, or 48 wherein said manipulating comprises loading said cells with antigenic peptides.

50. The method of claim 44, 45, 46, 47, or 48 wherein said manipulating comprises transfecting said cells with MHC genes and loading said cells with antigenic peptides.

51. The method of claim 44, 45, 46, 47, or 48 wherein said manipulating comprises coating said cells with pre-formed MHC:antigenic peptide complexes.

52. The method of claim 26, 44, 45, or 46 wherein said polypeptide is selected from the group consisting of polypeptides that comprise amino acid sequences from Fas

ligand, an anti-class I MHC heavy chain antibody, an anti-class I MHC heavy chain Fv chimeric polypeptide, an anti-β_2 microglobulin antibody, and anti-β_2 microglobulin Fv chimeric polypeptide, an anti-Fas antigen antibody, an anti-Fas antigen Fv chimeric polypeptide, interleukin-10, transforming growth factor β, heterodimeric placental protein 14, homodimeric placental protein 14, and an immunoregulatory viral protein.

53. The method of claim 47 or 48 wherein said secreted molecule is selected from the group consisting of interleukin-10, transforming growth factor β, an anti-class I MHC heavy chain antibody, an anti-class I MHC heavy chain Fv chimeric polypeptide, an anti-β_2 microglobulin antibody, an anti-β_2 microglobulin Fv chimeric polypeptide, an anti-Fas antigen antibody, an anti-Fas-antigen Fv chimeric polypeptide, a chimeric bacterial toxin molecule, HIV-tat, CTLA4Ig, a viral immunoregulatory protein, heterodimeric placental protein 14, homodimeric placental protein 14, or an antibody against a T-cell activation antigen.

54. The method of claim 8, 23, 28, 34, or 53 wherein said chimeric bacterial toxin comprises a cytokine that binds to a receptor on a T cell, an antibody that binds to an antigen on a T cell or a Fv molecule that binds to an antigen on a T cell.

55. The method of claim 54, wherein said chimeric bacterial toxin comprises the toxin domain from diphtheria toxin or Pseudomonas exotoxin.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/05187

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A61K 48/00
US CL :Please See Extra Sheet.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. :
514/44

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

None

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, Dialog
search terms: veto cells, T cell proliferation, chimera

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Kidney International, Volume 43, Supplement 39, issued 1993, Tykocinski et al., "Prospects for anti-rejection therapies based upon CD8-dependent immunoregulation", pages S-120-S-123, see entire reference.	1, 9, 11-15, 20, 26, 29, 31 and 32
Y	International Immunology, Volume 6, No. 10, issued 1994, Takahashi et al., "Human Fas ligand: gene structure, chromosomal location and species specificity", pages 1567-1574, see entire reference.	1, 9, 11-15, 20, 26, 29, 31 and 32
Y	US, A, 5,242,687 (Tykocinski et al) 07 September 1993, see entire document.	1, 9, 11-15, 20, 26, 29, 31 and 32

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
11 JULY 1996	07 AUG 1996
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer Suzanne Ziska, Ph.D. Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/05187

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Molecular Immunology, Volume 31, No. 13, issued 1994, Huang et al., "Alloantigenic Recognition of Artificial Glycosyl Phosphatidylinositol-Anchored HLA-A2.1", pages 1017-1028, see entire reference.	1, 9, 11-15, 20, 26, 29, 31 and 32
Y	Nature, volume 370, issued 25 August 1994, Lowin et al., "Cytolytic T-cell cytotoxicity is mediated through perforin and Fas lytic pathways", pages 650-652, see entire reference.	1, 9, 11-15, 20, 26, 29, 31 and 32
Y	Cell, Volume 76, issued 28 January 1994, Germain, R.N., "MHC-Dependent Antigen Processing and Peptide Presentation: Providing Ligands for T Lymphocyte Activation", pages 287-299, see entire reference.	1, 9, 11-15, 20, 26, 29, 31 and 32

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/05187

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3 ☐ Claims Nos..
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3 ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1, 9, 11-15, 20, 26, 29, 31 and 32

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/05187

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

514/44

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I, claim(s) 1, 9, 11-15, 20, 26, 29, 31 and 32, drawn to a method for producing an artificial veto cell capable of specifically inhibiting the proliferation, cytotoxicity or cytokine secretion comprising the steps of providing an antigen presenting cells presenting an alloantigen and externally contacting the extracellular surface of said APC with a chimera comprising a non-CD8 polypeptide and a moiety sufficient to bind the chimera to the cell surface in a manner which presents the polypeptide such that the polypeptide is capable of reducing T cell proliferation, the method of making the artificial veto cell and the first claimed method of using the artificial veto cell.

Group II, claim(s) 2-5, 8-12, 16-18 and 54, drawn to a method for producing an artificial veto cell differing from the method of group I in that the APC is genetically transformed to express the non-CD8 polypeptide.

Group III, claim(s) 6, 9 and 12-15, drawn to a method for producing a non-naturally occurring biological membrane derived from an artificial veto cell by isolating the membrane and then treating the membrane.Group IV, claims 7 and 9-15, drawn to amethod for producing a non-naturally occuring biological membrane derived from a genetically transformed artificial veto cell by transfecting the cell and then isolating the membrane.

Group V, claim 19, drawn to a GPI modified polypeptide.

Group VI, claims 21-23 and 54, drawn to an artifical veto cell bearing a transfected genetic sequence.

Group VII, claims 24 and 25, drawn to an artificial veto cell containing an exogenously added secreted molecule capable of reducing T cell proliferation.

Group VIII, claims 27-29 and 54, drawn to an in vitro method for inhibiting an antigen-specific T cell comprising providing an artificial veto cell which locally releases a secreted molecule and has an MHC:nominal antigen peptide complex on its surface and exposing T cells capable of responding to said complex so that the secreted molecule inhibits the cellular immune response of said T cells to said antigen.

Group IX, claims 27, 30-34 and 54, drawn to an in vivo method of inhibiting an antigen specific T cell response.
Group X, claims 35, 38 and 41-43, drawn to a membrane associated anti-class I MHC antibody comprising an immunoglobuling heavy chain polypeptide and an immunoglobulin light chain polypeptide.
Group XI, claims 36, 39 and 41-43, drawn to a membrancassociatedanti-β2 microglobulin antibody.

Group XII, claims 37 and 40-43, drawn to a membrane associated anti-Fas antibody.

Group XIII, claims 44 and 49-52, drawn to a method for producing an artificial veto cell comprising manipulating the cell so that an alloantigen or processed antigen is presented on the extracellular surface.

Group XIV, claims 45-55, drawn to a method for producing a genetically engineered artificial veto cell comprising manipulating the cell so that an alloantigen or processed antigen is presented on the extracellular surface.

The inventions listed as Groups I-XIV do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: The first claimed product, methods of making the first claimed product and methods of using the first claimed product all encompass the same special technical feature which is the particular artificial veto cell having an antigen prebound to the cell surface wherein the extracellular surface of the cell is contacted with a chimera containing a non-CD8 polypeptide linked to a moiety sufficient to bind the chimera to the cell surface. The other inventions do not contain the special technical feature which is the first claimed artificial veto cell having the specific characteristics. Other veto cells having other cell surface characteristics would have different biological features and responses when used either in vivo

or in vitro and therefore differ biologically from the veto cell of the first claimed method in claim 1. Other inventions are directed to different veto cells, different antibodies, polypeptides, and different methods of making various veto cells and do not contain the special technical feature of Invention I.